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ORIGINAL PAPER

A comparative study on heavy metal content of plants irrigated with tap and wastewater

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Abstract The objective of the present study was to compare the potential accumulation capacity of Cr, Cu and Zn in *Zea mays* and *Populus deltoides* Marsh. The study was conducted in an area in a wastewater treatment plant in Birjand for a period of 120 days. The soil sample and selected parts of *Z. mays* and *P. deltoides* Marsh were collected monthly and treated with hydrochloric acid and nitric acid. The results showed that the highest mean concentrations of Cu (1.78 ± 0.93 mg kg^{-1}) and Zn (2.65 ± 1.37 mg kg^{-1}) were observed at *Z. mays* in September. The results indicated that the concentrations of Cu and Zn in *Z. mays* and *P. deltoides* Marsh shoots irrigated with wastewater were higher than tap water. In addition, both the plants comparatively showed a significant increase in total metal amount when treated with wastewater ($p < 0.001$). The study of BCF and TF demonstrated that *Z. mays* was suitable for phytoextraction of Cu, but unsuitable for both phytoextraction and phytostabilization of Zn and Cr, whereas *P. deltoides* Marsh was unsuitable for both phytoextraction and phytostabilization of Zn, Cu and Cr.

Keywords *Zea mays* · *Populus deltoides* Marsh · Phytoextraction · Phytostabilization

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Introduction

Phytoremediation is the utilization of vegetation to remove, replace, stabilize and decrease pollutants in soil, sediment and water (Padmavathiamma and Loretta 2007). The most essential reasons for successful phytoremediation utilization are the site conditions, anticipated land use and the types of plants (Thangavel and Subhuram 2004).

Large volume of water is being consumed in farming, industry, domestic and civic usage. Agriculture is the largest user of freshwater in the world (Sayadi et al. 2010; Sayyed and Sayadi 2011). Usage of wastewater for irrigation in agricultural activities causes the biomagnification and accumulation of heavy metals in food chain (Akpor and Muchie 2010; Sayadi and Rezaei 2014). At the present scenario, the main challenge is how to handle the waste which is released at a faster rate as compared to its suitable removal (Madyiwa et al. 2002). Harvest irrigation by wastewater increases concerns about the safety of vegetables because their heavy metal content is increased (Muchuweti et al. 2006; Zojaji et al. 2014). Cu causes several physiological processes in florae, including photosynthesis, respiration, carbohydrate spreading, nitrogen and cell wall metabolism, seed production and disease resistance. Moreover, the higher concentration may cause to reduction of root growth and leaf chlorosis (Shabani and Sayadi 2012).

Cr not only weakens enzyme performance and plant growth, but also leads to chlorosis, membrane and root damage (Khan et al. 2006). According to Cardwell et al. (2002), aquatic plants have been widely utilized to clean polluted waters. In that research, copper, cadmium, lead and zinc were selected as the metals for the study because the existence of cadmium above trace levels in the environment is an indicator of pollution and lead is a common contaminant from road runoff. Zinc is a common metal existing in variable amounts and, if found in significant amounts, can be an indicator of industrial pollution. Moreover, copper is an indicator of industrial contamination of urban waters.

Ebbs and Kochian (1997) considered the poisonousness of Zn and Cu in three species from Brassica genus, viz. *B. juncea, B. rapa* and *B. napus*. They reported that Brassica species were more effective at removing zinc from nutrient solution than Cu. Besides, the quantity of Zn and Cu removal was reduced when both metals were present. Ciura et al. (2005) explored the possibility of using common crops (red beet, field pumpkin, chicory, common bean, barley, white cabbage, maize, alfalfa, common parsnip) to remove heavy metals from the soil. They suggested that the most effective crop in the extraction of Cd, Mn, Cu, Ni, Pb and Zn was pumpkin. Likewise, most effective crops in Cr and Fe extractions were maize and alfalfa, respectively. The main objective of this study was a comparative accumulation capacity of Cr, Cu and Zn in *Zea mays* and *Populus deltoides Marsh* cultivated on contaminated sites. The research was carried out in an area of wastewater treatment plant in the city of Birjand in 2012.

Materials and methods

Zea mays and *P. deltoides* Marsh were cultured in two separate areas constituting a total area of 4 m^2. The experimental plants were irrigated with tap water and wastewater for 4 subsequent months, viz. August to November 2011. The irrigation was carried until it reached the field capacity. Five grams of samples of the root, stem and leaf of *Z. mays* and *P. deltoides* Marsh were assembled once a month. The collected samples were washed with distilled water, dried, and ashed in an oven at 600 °C for 6 h. Triplicate sets of dehydrated samples were broken down with 5 cc acid, and volume of the watery digested samples were made up to 25 ml (Schmidt 2003). 0.5 kg soil samples of soil at 0–30 cm depth were collected in triplicate sets and combined. To achieve the bio-available concentration of the metals, the soil samples were dried at 105 °C and filtered through a 2-mm sieve. Then, 2 g soil samples were mixed with 5 ml of hydrogen peroxide (30 %) and digested at room temperature for 1 h with occasional shaking. A second 5 ml aliquot of hydrogen peroxide was introduced into and digested at 85 °C (water bath) for 1 h. The contents were evaporated to a small volume (1–2 ml). Twenty-five milliliters of ammonium acetate (0.1 ml/l, adjusted to pH 2 with nitric acid) was added to the cool and moist residue. The volume of the watery digested samples was made up to 25 ml and introduced into the water bath for 12 h (Radojevic and Bashkin 1999). The digested samples were assessed in the Water and Wastewater Company, South Khorasan, using AA-7000 series of atomic absorption (Shimadzu) by flame mode, and concentration of Zn, Cr and Cu was evaluated. The transfer factor is the motion of a heavy metal from the root to the aerial parts (stem and leaves), which was assessed using the subsequent formula (Kim et al. 2003):

$$TF = \frac{\text{Element of Shoots (stem and leaves) (mg kg}^{-1})}{\text{Element of root mg kg}^{-1}} \times 100$$

TF < 1 indicate that the metals are stored in the roots of a plant, and TF > 1 shows translocation of metals to the shoots of plants. Metal uptake is also referred as a bio-concentration factor (BCF). It illustrates the capability of a plant to accumulate a specific metal from the soil substrate (Zayed et al. 1998). It was calculated as follows:

$$BCF = \frac{\text{Average Heavy Metal concentration in root (mg kg}^{-1})}{\text{Heavy Metal added in soil (mg kg}^{-1})}$$

The zero BCF value implies limited movement of metals from sediment to a plant. The higher BCF value is more suitable for phytoextraction (Blaylock et al. 1997). However, BCF > 2 relates a high value. Nevertheless, a change in BCF is related to the individual plant biomass and soil elemental focus, and the productivity of BCF is better understood when it is compared between different plant species or elements.

According to Yanqun et al. (2005), enrichment factor (EF) is defined as the concentration of heavy metals in plant shoots divided by the heavy metal concentration in soil, whereas Khan et al. (2006) reported that enrichment coefficient depends on the soluble portion of metals and organic matters in soils:

$$EF = \frac{\text{Average Heavy Metal concentration in shoot (mg kg}^{-1})}{\text{Heavy Metal concentration in soil (mg kg}^{-1})}$$

Statistical analysis

All experiments were carried out in triplicate, and significant differences were analyzed by ANOVA (Mann–Whitney, *U* test due to nonparametric data) using SPSS software.

Results and discussion

Cr, Cu and Zn concentrations in soils

The wastewater treatment plot was composed of sand (39 %), silt (48 %) and clay (13 %). The concentration of Cr in soils irrigated with wastewater and tap water was 2.146 and 2.005 mg kg^{-1}, respectively. The Cu

Table 1 Concentration of Cr, Cu and Zn in different parts of *Zea mays* and *Populus deltoides* Marsh (mg kg^{-1})

Plant	Treatment	Portion of plant	November			October			September			August		
			Zn	Cu	Cr	Zn	Cu	Cr	Zn	Cu	Cr	Zn	Cu	Cr
Zea mays	Tap water	Root	1.69	1.13	2.37	2.64	1.65	2.21	4.27	1.6	2.12	3.28	0.9	1.13
		Stem	1.22	1.41	0.16	1.01	1.51	0.2	2.24	1.2	0.18	1.68	0.75	0.26
		Leaves	0.62	0.44	0.24	1.49	0.76	0.22	0.8	0.87	0.14	0.58	0.61	0.17
	Wastewater	Root	1.74	1.15	2.54	2.69	1.67	2.47	4.27	2.12	2.12	3.28	2.87	1.37
		Stem	1.34	1.48	0.36	1.06	1.68	0.3	2.26	1.44	0.29	1.77	1.37	0.28
		Leaves	0.69	1.47	0.42	1.6	1.84	0.38	2.03	3.47	0.35	4.38	3.28	0.33
		Mean	1.22	1.18	1.02	1.75	1.52	0.96	2.65	1.78	0.87	2.50	1.63	0.59
		Max	1.74	1.48	2.54	2.69	1.84	2.47	4.27	3.47	2.12	4.38	3.28	1.37
		Min	0.62	0.44	0.16	1.01	0.76	0.20	0.80	0.87	0.14	0.58	0.61	0.17
		SD	0.48	0.39	1.12	0.75	0.39	1.07	1.37	0.93	0.97	1.39	1.16	0.52
Populus deltoides Marsh	Tap water	Root	1.28	0.59	0.54	1.79	0.32	0.44	1.51	2.15	0.24	1.45	1.45	0.69
		Stem	1.69	0.59	0.13	2.13	0.39	0.14	1.71	1.77	0.16	1.91	1.32	0.15
		Leaves	1.15	0.45	0.2	1.19	0.37	0.21	0.95	0.51	0.18	1.26	0.22	0.18
	Wastewater	Root	1.38	0.63	0.57	1.8	0.38	0.54	1.62	2.15	0.57	2.43	1.58	0.76
		Stem	2.37	0.68	0.15	3.41	0.55	0.15	2.9	1.99	0.16	4.36	1.49	0.22
		Leaves	1.44	0.55	0.26	1.26	0.43	0.22	1.38	0.56	0.2	1.34	0.38	0.19
		Mean	1.55	0.58	0.31	1.93	0.41	0.28	1.68	1.52	0.25	2.13	1.07	0.37
		Max	2.37	0.68	0.57	3.41	0.55	0.54	2.90	2.15	0.57	4.36	1.58	0.76
		Min	1.15	0.45	0.13	1.19	0.32	0.14	0.95	0.51	0.16	1.26	0.22	0.15
		SD	0.44	0.08	0.20	0.81	0.08	0.17	0.65	0.78	0.16	1.18	0.61	0.28

concentration in soils irrigated with wastewater and tap water was 1.23 and 0.899 mg kg^{-1}, respectively. Furthermore, Zn concentration in soils irrigated with wastewater and tap water was 4.16 and 2.54 mg kg^{-1}, respectively.

Cr, Cu and Zn concentrations in wastewater and tap water

The concentration of Cr in wastewater that is provided from wastewater treatment plant of Birjand City and tap water was 12 and 5 mg l^{-1}, respectively. In addition, the concentrations of Cu and Zn in wastewater and tap water were 6.5 and 2 and 48.2 and 21.35 mg l^{-1}, respectively.

Concentrations of Cr, Cu and Zn in the plants

The results of the present study showed that the average concentrations of Cr in the roots of *Z. mays* and *P. deltoides* Marsh irrigated with wastewater and tap water were 2.13 and 1.96 and 0.61 and 0.48 mg kg^{-1}, respectively. The average concentrations of Cu in the roots of *Z. mays* and *P. deltoides* Marsh irrigated with wastewater and tap water were 1.95 and 1.32 and 1.19 and 1.13 mg kg^{-1}, respectively. And the average concentrations of Zn in the roots of *Z. mays* and *P. deltoides* Marsh irrigated with wastewater and tap water were 3.00 and 2.97 and 1.81 and 1.51 mg kg^{-1}, respectively.

The highest Cr concentration was measured in the root of *Z. mays* (2.54 mg kg^{-1}) treated with wastewater in November, while the lowest Cr concentration was detected in the stem of *P. deltoides Marsh* (0.13 mg kg^{-1}). Comparatively, the concentrations of Cr in shoots and roots of *Z. mays* and *P. deltoides* Marsh irrigated with wastewater were higher than those of the plants irrigated with tap water (Table 1).

The highest Cu concentration was measured in the sample of *Z. mays* (3.47 mg kg^{-1} in leaves), while the lowest concentration was detected in the sample of *P. deltoides* Marsh (0.22 mg kg^{-1} in leaves). The concentrations of Cu in *Z. mays* and *P. deltoides* Marsh shoots irrigated with wastewater were comparatively higher in plants irrigated with tap water (Table 1).

The highest Zn concentration was measured in the sample of *Z. mays* (4.38 mg kg^{-1} in leaves), while the lowest concentration was detected in the sample of *P. deltoides* Marsh (0.58 mg kg^{-1} in leaves). The concentrations of Zn in *Z. mays* and *P. deltoides* Marsh shoots irrigated with wastewater were higher than those in plants irrigated with tap water (Table 1).

Metal concentration in the treatments

Total Cr, Cu and Zn concentrations were measured in varied parts of each plant at the tap water and wastewater

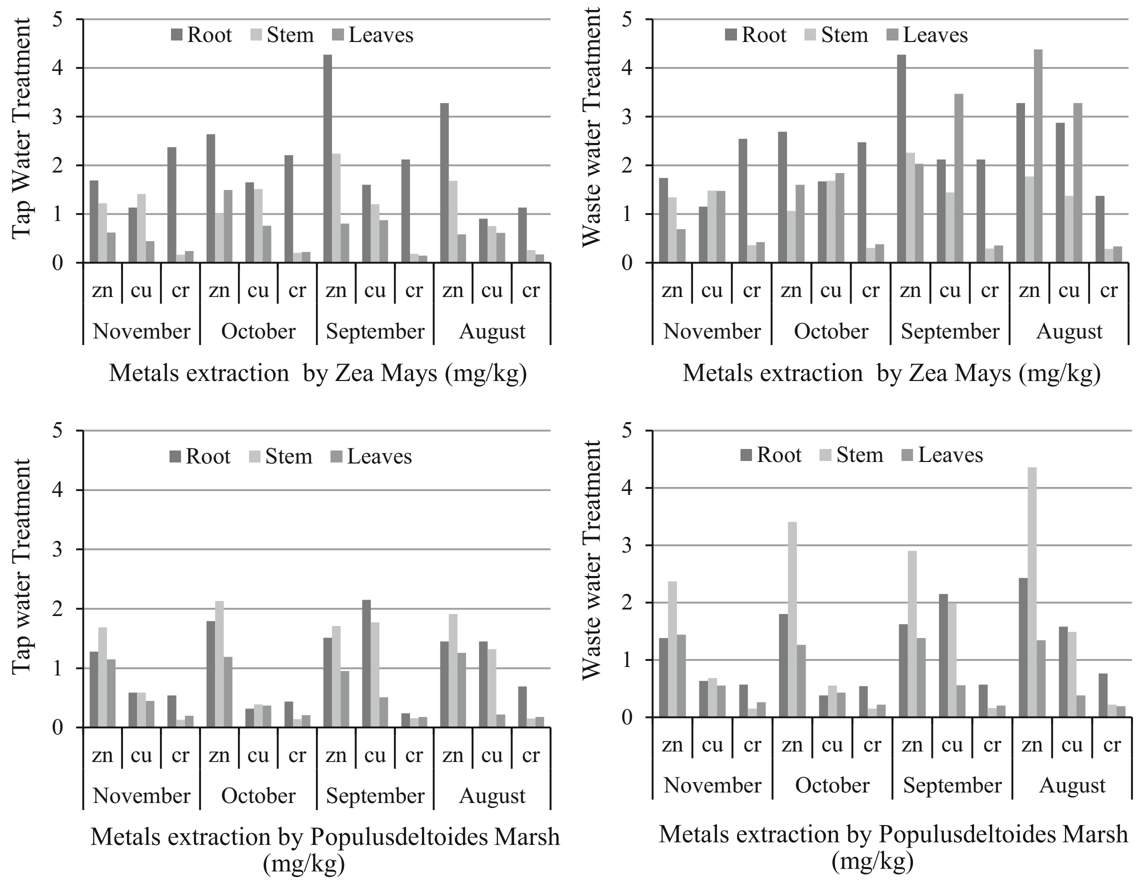

Fig. 1 Comparison of metal accumulation in root, stem and leaves of *Zea mays* and *Populus deltoides* Marsh treated for 4 successive months with tap water and wastewater, respectively

treatments. Comparative metal accumulation in the root, stem and leaves of *Z. mays* and *P. deltoides* Marsh for 4 successive months treated with tap water and wastewater treatment is presented in the Fig. 1. With few exceptions, the higher metal concentration was observed in the roots of *Z. mays,* whereas in *P. deltoides* Marsh, higher metal concentration was observed in stems (Fig. 1). The two plants showed a significant increase in total metal content in the wastewater treatment as compared to the tap water treatment plot ($p < 0.001$). As far as, the metal concentration in roots, stems and leaves is concerned, it exhibited a significant difference between the two experimental plants ($p < 0.05$).

Cr, Cu and Zn translocation factor (root/shoot)

The translocation ability of these heavy metals in *Z. mays* was in the following order: Cu (2.06) > Zn (1.26) > Cr (0.31), while the translocation ability of these heavy metals in *P. deltoides* Marsh was Zn (2.55) > Cu (1.39) > Cr (0.64). Metals that are absorbed by plants and mostly stored in the roots of plants are indicated by TF value <1. The translocation value >1 indicates translocation to the shoots of a plant. In the present study, translocation value >1 for Zn and Cu were found in *Z. mays* and *P. deltoides* Marsh, while translocation values for Cr in the *Z. mays* and *P. deltoides* Marsh were <1 (Fig. 2). TF higher than 1 indicated an effective ability to transport metal from root to leaf, most probably via an efficacious metal transporter system of plants (Zhao et al. 2002). It could be even sequestration of metals in leaf vacuoles and apoplast (Lasat et al. 2000). Translocation factor is an indication of the plant capability to translocate metal from roots to the aerial parts (Marchiol et al. 2004). As indicated in Fig. 1, it can be assumed that these metals (Zn and Cu) are transported to the aerial parts of the plant, while Cr are stored in the roots. Yanqun et al. (2005) stated that species with TF < 1 were excluder species. Thus, it can be expected that *Z. mays* and *P. deltoides* Marsh are excluder species for Cr. Comparative study of TF in *Z. mays* and *P. deltoides* Marsh *indicated* that translocation factor of Zn and Cr in *P. deltoides* Marsh is greater than that in *Z. mays,* whereas TF of Cu in *Z. mays* is greater than that in *P. deltoides* Marsh (Fig. 1). Figure 1 shows that *P. deltoides* Marsh had a greater transport of Zn and Cr from root to shoot in comparison to *Z. mays,* while *Z. mays* exhibited a greater transport of Cu

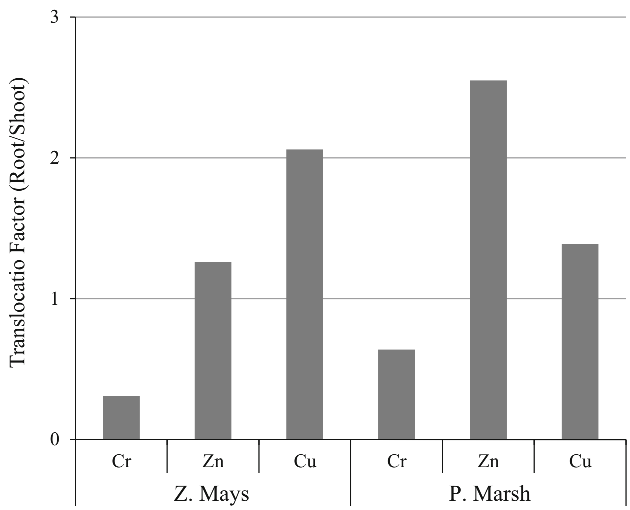

Fig. 2 Translocation factor of Cr, Cu and Zn in *Zea mays* and *Populus deltoides* Marsh

from root to shoot in comparison to *P. deltoides* Marsh. It is interesting to note that the maximum transport was determined in *P. deltoides* Marsh for Zn and that the minimum transport was detected in *Z. mays* for Cr.

Cr, Cu and Zn bio-concentration factor

Figure 3 shows the BCF movement of each metal from soil to *Z. mays* and *P. deltoides* Marsh plants. However, zero BCF value implies limited movement from the soil to the plant. BCF value of *Z. mays* at the root was highest for Cu (1.58) followed by Cr (0.99) and Zn (0.72), while the stem and leaf values were in the following order Cu > Zn > Cr. The BCF of *Z. mays* for Zn and Cr in root, stem and leaf showed low value <1, and BCF of *P. deltoides* Marsh for Zn, Cu and Cr in root, stem and leaf showed low value <1 (Fig. 3).Thus, this indicated a limited movement of metals from soil to the plant. According to Ghosh and Singh (2005), BCF is an index of plant capability to accumulate a particular metal with respect to its concentration in the sediment. The BCF of *Z. mays* for Cu in root and stem was >1. The BCF value >1 showed that plant is suitable for phytoextraction (Blaylock et al. 1997).

Plants with both BCF and translocation factor greater than one (TF and BCF >1) have the potential to be utilized in phytoextraction, whereas plants with BCF > 1 and TF < 1 have the potential for phytostabilization (Yoon et al. 2006).

The results of the present study revealed that *Z. mays* is suitable for phytoextraction of Cu and is unsuitable for both phytoextraction and phytostabilization of Zn and Cr. Additionally, *P. deltoides* Marsh is unsuitable for both phytoextraction and phytostabilization of Zn, Cu and Cr.

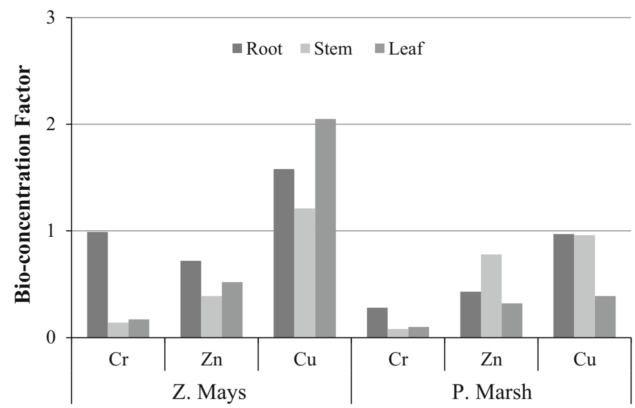

Fig. 3 Bio-concentration factor in the root, stem and leaf of *Zea mays* and *Populus deltoides* Marsh

Cr, Cu and Zn enrichment coefficient (EC)

Enrichment coefficient is indicating phytoremediation of species (Zhao et al. 2003). Both EF and TF have to be measured in order to evaluate whether a particular plant is a metal hyperaccumulator or not. Therefore, a hyperaccumulator plant should have EF > 1 and TF > 11, and a total accumulation >1,000 mg kg^{-1} of Cu, Cr, Co, Ni or Pb and/or >10,000 mg kg^{-1} of Fe, Mn or Zn (Ma et al. 2001). In this study, the enrichment coefficient of Cu in *Z. mays* was >1.0, while EC of Cr and Zn was <1.0 (Table 2). Moreover, EF of Cu and Zn in *P. deltoides* Marsh was >1.0, and EF of Cr was <1.0. Therefore, it can be safely said that *Z. mays* can be regarded as a hyperaccumulator of Cu, while *P. deltoides* Marsh can be regarded as a hyperaccumulator of Cu and Zn.

Conclusion

The identifications of plants play a key role in phytoremediation for management of contaminated soil and water. This study was conducted in an area integrated with natural conditions where the effect of soil buffering capacity influenced nutrient accessibility to a plant. Though the Cr, Cu and Zn uptake and transfer to the different parts of a plant are not exactly understood, but the behavior of the plant remains fairly consistent across the site. In the present study, both the experimental plants showed a significant increase in total metal amount when treated with the wastewater as compared to the tap water treatment ($p < 0.001$). Besides, the study of BCF and TF demonstrated that *Z. mays* was suitable for phytoextraction of Cu and unsuitable for both phytoextraction and phytostabilization of Zn and Cr, whereas *P. deltoides* Marsh was unsuitable for both phytoextraction and phytostabilization

Table 2 Enrichment coefficient of Cr, Cu and Zn in *Zea mays* and *Populus deltoides*

Heavy metals	Plant	
	Z. mays	*P. deltoides*
Cr	0.31	0.18
Cu	3.26	1.35
Zn	0.91	1.11

of Zn, Cu and Cr. The study of EF and TF indices showed that *Z. mays* can be regarded as a hyperaccumulator of Cu and that *P. deltoides* Marsh can be considered as a hyperaccumulator of both Cu and Zn. Thus, it could be safely concluded that biomass production, growth rate and soil characteristics, etc. strongly influenced phytoremediation, and therefore, further studies on these experimental plants are highly suggested.

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